Plasticity in skeletal characteristics of nursery-raised staghorn coral, *Acropora cervicornis*

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Abstract

Staghorn coral, *Acropora cervicornis*, is a threatened species and the primary focus of western Atlantic reef restoration efforts to date. We compared linear extension, calcification rate, and skeletal density of nursery-raised *A. cervicornis* branches reared for six months either on blocks attached to substratum or hanging from PVC trees in the water column. We demonstrate that branches grown on the substratum had significantly higher skeletal density, measured using computerized tomography, and lower linear extension rates compared to water-column fragments. Calcification rates determined with buoyant weighing were not statistically different between the two grow-out methods, but did vary among coral genotypes. Whereas skeletal density and extension rates were plastic traits that depended on grow-out method, calcification rate was conserved. Our results show that the two rearing methods generate the same amount of calcium carbonate skeleton but produce colonies with different skeletal characteristics, and suggest that there is genetically based variability in coral calcification performance.

Introduction

Throughout the last 12,000 yr in the western Atlantic, *Acropora* spp. corals created entire reef zones (Goreau 1959), were among the most prolific with respect to reef carbonate production (Macintyre et al. 1977; Hubbard 2013), and generated critical habitat for fish assemblages (Alevizon and Porter 2015). After their near-complete loss from many areas of the Caribbean, primarily as a result of white-band disease (Aronson and Precht 2001), populations of *Acropora* were reduced to a fraction of what they were prior to the 1980s (Precht et al. 2004). The listing of *A. cervicornis* and *A. palmata* as threatened under the U.S. Endangered Species Act in 2006

led to efforts to revive populations in U.S. waters and elsewhere in the region (NMFS, NOAA 2006).

In response to low levels of natural *Acropora* recovery, over 150 ocean-based nursery programs have been established across the western Atlantic since 2000, with the majority concentrating on *A. cervicornis* (Lirman and Schopmeyer 2016). Recent evidence suggests that out-planting of *A. cervicornis* from nurseries is successfully increasing populations on a reef-scape scale in the Florida Keys (Miller et al. 2016), providing impetus to expand reef restoration efforts even further. Most nursery programs focus resources on fragment production and out-planting, and have not quantitatively examined the efficacy of different grow-out techniques. Nurseries and field experiments that have included monitoring coral growth rates have traditionally focused on measuring linear extension of colonies in various ways (e.g., Shinn 1966; Lirman et al. 2014), but directly measuring calcification rates using the buoyant weight technique (Jokiel et al. 1978) is gaining in popularity (Kuffner et al. 2013; Morrison et al. 2013; Lohr and Patterson 2017). Calcification is the fundamental process by which corals precipitate calcium carbonate. Decades of research on coral calcification have revealed that environmental variables including temperature, solar irradiance, water motion, nutrients, and aragonite saturation state influence calcification rates (reviewed by Buddemeier and Kinzie 1976; Jokiel et al 2016). As part of a larger, ongoing study measuring spatial and temporal variability in coral calcification rates throughout the Florida Keys (Kuffner et al. 2013), we tested the hypothesis that coral calcification rates, linear extension, and skeletal density varied between two widely adopted grow-out techniques: *A. cervicornis* fragments attached to blocks on the ocean bottom, and fragments hanging from monofilament line suspended in the water column from polyvinyl chloride (PVC) trees. Anecdotal evidence and discussion with coral-restoration practitioners lead

us to hypothesize that fragments raised on trees would have greater linear extension and calcification rates, but possibly less dense skeletons than those raised attached to the substratum.

Methods

Acropora cervicornis branch tips were harvested from coral colonies in cultivation at Mote Marine Laboratory's offshore nursery located near Looe Key Sanctuary Preservation Area of the Florida Keys National Marine Sanctuary (24°33.765'N, 81°24.008'W). Fragments of ten unique genotypes (Baums et al. 2005, 2010) were snipped from nursery colonies and fixed to ¼-inch thick, 3-inch diameter PVC discs using All-fix epoxy putty (CirCut Corporation, Lafayette Hill, Pennsylvania, U.S.A.) (Fig. 1a), or attached to 100-pound-test monofilament line with a numbered aluminum tag (Fig. 1b). For the first method, a 3-inch stainless-steel hex-head bolt placed through a hole in the plate (held in place by the epoxy) was used to hold a PVC end cap that slid over PVC pipe attached to cement blocks at the Mote nursery (Fig. 1c). Not all replicates survived the mounting process, resulting in twelve PVC-disc corals (treatment = "block") representing eight genotypes and nine monofilament-line corals representing seven of the same and an additional two genotypes (Fig. 1d, treatment = "water column"). Block and water-column corals were placed back at the Mote nursery at 7.9 and 6.4 m water depth, respectively, on 20 April 2015.

The corals grew at the nursery until they died during the late-summer 2015 bleaching event. Paling was first observed on 29 July, and by 6 October all fragments in both treatments were completely bleached but none were yet dead. By 28 October, all fragments were dead (as evidenced by bare or nearly bare skeletons), and corals were removed from the nursery between 14 and 28 October and weighed on 30 October. Coral calcification rates were measured using the

buoyant-weight technique (Jokiel et al. 1978; Morrison et al. 2013) by subtracting their weight in seawater at the start of the experiment from their weight after 191 d (19 April to 30 October 2015) and calculating dry weight gained d^{-1} (mg d^{-1}) using a seawater density of 1.02 g cm^{-3} and an aragonite density of 2.93 g cm^{-3} resulting in a conversion equation of $Wair = 1.53Wwater$ (Jokiel et al. 1978). Because we used single branch tips of approximately equal size at the beginning of the study (≈1-cm^2 planar footprint if standing on end, mean ± SD diameter of branch tip: 1.2 ± 0.14 cm, original tip height: 6.8 ± 1.7 cm), calcification rates were not normalized to any measure of surface area or initial weight. Original branch-tip length and net total linear extension (TLE) were measured to the nearest 0.1 mm using calipers (Johnson et al. 2011). Skeletal density of the corals (Figs. 1e and 1f) was measured by computerized tomography (CT) using a Siemens Somatom Volume Zoom CT scanner. Density of skeleton added during the experimental period was determined using the Amira software package (FEI Company, Inc.) by digitally bisecting the CT image stack at the measured original height of each fragment. Mean of brightness of the entire volume of new growth was converted to real-world skeletal density using aragonite density phantoms.

Data were analyzed using the software package Statistix 10. Two-sample Student's t-tests were used to compare skeletal response variables between rearing methods, and two-way analysis of variance (ANOVA) with treatment and genotype as the two main factors was used to compare calcification rates among genotypes for which there were at least two fragments (n = 8). When the assumption of equal variance among groups was violated for a t-test, Satterthwaite's two-sample t-test assuming unequal variance was reported instead. Our data met the assumptions of the ANOVA model including normality of residuals and homogeneity of variance.

Results and discussion

After six months of growth, *A. cervicornis* branches reared on lines in the water column grew significantly longer (net TLE) than those on blocks (mean ± SD for blocks = 9.98 ± 4.48 cm, water column = 15.0 ± 5.00 cm; two-sample t-test: t = 2.4, p = 0.024; Fig. 2a). Number of branches produced by the original branch did not significantly vary between the grow-out methods (blocks = 3.7 ± 2.6 branches, water column = 5.0 ± 2.8 branches, Two-sample t-test: t = 1.1, p = 0.28, power of detecting two-branch difference = 0.36). Conversely, skeletal density was significantly greater in colonies on blocks compared to those reared in the water column (blocks = 1.25 ± 0.10 g cm^{-3}, water column = 1.10 ± 0.05 g cm^{-3}; Satterthwaite's two-sample t-test assuming unequal variance t = 4.4, p = 0.0004; Fig. 2b). Calcification rate was not statistically different between the two rearing methods (blocks = 43.9 ± 15.2 mg d^{-1}, water column = 51.8 ± 16.3 mg d^{-1}; two-sample t-test: t = 1.6, p = 0.26; Fig. 2c), though power of the t-test to detect the observed difference in treatment means was low (= 0.19). Interestingly, there was an unexpectedly pronounced effect of coral genotype on calcification rate, given the low number of replicates per genotype, regardless of grow-out method (two-way ANOVA, grow-out treatment effect: F = 0.44, p = 0.52, genotype effect: F = 9.9, p = 0.0008; Fig. 3). All data are available at https://doi.org/10.5066/F7HH6H72.

Our comparison of two common methods used to rear *A. cervicornis* fragments in nurseries revealed that, whereas the skeletal density was higher on blocks and linear extension was greater on trees, the colony-specific rate of calcification did not significantly differ. A similar study that tested the tree vs. block grow-out methods on *A. cervicornis* also found that the tree method resulted in much higher TLE (O'Donnell et al. 2017). Although they did not measure skeletal density, O'Donnell et al. (2017) hypothesized that corals grown on blocks may

divert more calcification effort to fortifying their skeletons because of the greater force exerted by water currents on stationary corals compared to those moving with the current on trees. CT scanning revealed that our block corals were indeed denser, and we suggest that coral density and linear extension rate may be more malleable growth traits (i.e., more responsive to environmental forces) compared to calcification rate (more genetically controlled). Coral calcification is an energetically expensive process (Cohen et al. 2003), so our results suggest that the corals in both treatments may have optimized the use of their similarly limited energy stores to grow in a morphology that was best suited for the environment they were experiencing. We did not measure food availability or incident light in our experiment, but we hypothesize that they were similar between the grow-out treatments since the groups of corals were within a few meters of each other in depth and horizontal space.

Because the total production of calcium carbonate material was similar between grow-out methods, employing both methods within a single nursery could maximize the versatility of fragments. Denser, stouter fragments raised on blocks would be better matched to high-energy environments (e.g., reef crest), and would break less under hydrodynamic stress (Enochs et al. 2014) and possibly have better survivorship, since dislodged colonies would likely be transported off the reef. Conversely, if the focus of a nursery was to maximize the production of coral tissue and calmer, back-reef or deeper fore-reef environments were targeted for restoration, the water-column method could be more advantageous. We conclude from our results that because linear extension rate and skeletal density are plastic traits, any newly produced skeleton originating from a fragment will adopt a growth form appropriate to its new environment. However, the capacity for skeletal plasticity does vary among coral species (Foster 1979), so testing of other species raised in nureries is recommended.

Given the small sample size within genotype (n = 2 to 3), the results presented in Fig. 3 suggesting genetic consistency in calcification rate regardless of rearing method are quite remarkable. Faster-growing *A. cervicornis* genotypes were also identified in recent work that used linear extension as the metric for coral-growth performance (Lirman et al. 2014; O'Donnell et al. 2017). In partial agreement with our results, a recent study on *A. cervicornis* found significant genotype effects for both TLE and calcification measured by buoyant weight (Lohr and Patterson 2017). Studies on other species have shown genotypic effects on branching morphology in *Pavona cactus* in Australia (Willis and Ayre 1985) and source-population effects on calcification rates in *Porites lobata* in American Samoa (Smith et al. 2007). Evidence for high levels of genetic diversity within and among populations of *A. cervicornis* along the Florida reef tract are mounting (Drury et al. 2016), and there is habitat-related variability in zooxanthellae clades associating with host corals (Baums et al. 2010). The results of our study using limited replication within genotypes suggest further testing of correspondence among calcification performance, host genotype, habitat, and zooxanthellae clades could provide valuable information to coral-restoration practitioners (Baums 2008). Calcification rate is fundamental to the occupation of space and fortification of the coral skeleton, and thus is likely related to survival rates, but further investigation into genotype performance once fragments are out-planted to the reef is recommended. The possibility that genetic influence on calcification rate may at times override the effect of growth environment highlights the importance of preserving and restoring both genetic and genotypic diversity in this threatened species of coral. It also adds to the hope that ecologically relevant levels of genetic and genotypic variability in an important coral trait may still exist in extant *A. cervicornis* populations in the Florida Keys upon which natural selection can act (Drury et al. 2016). While calcification rate is laborious to measure

because the corals need to be brought to shore for buoyant weighing, it may be a metric worth time investment during initial genotype selection when establishing a new nursery. Nonetheless, the discovery that calcification rate may be the genetically controlled growth-related trait, whereas linear extension and density are more responsive to environment, suggests that further investigation into the influence of both genetics and environment on all three of these metrics of *A. cervicornis* growth could enhance the efficacy of restoration efforts for this threatened species of coral.

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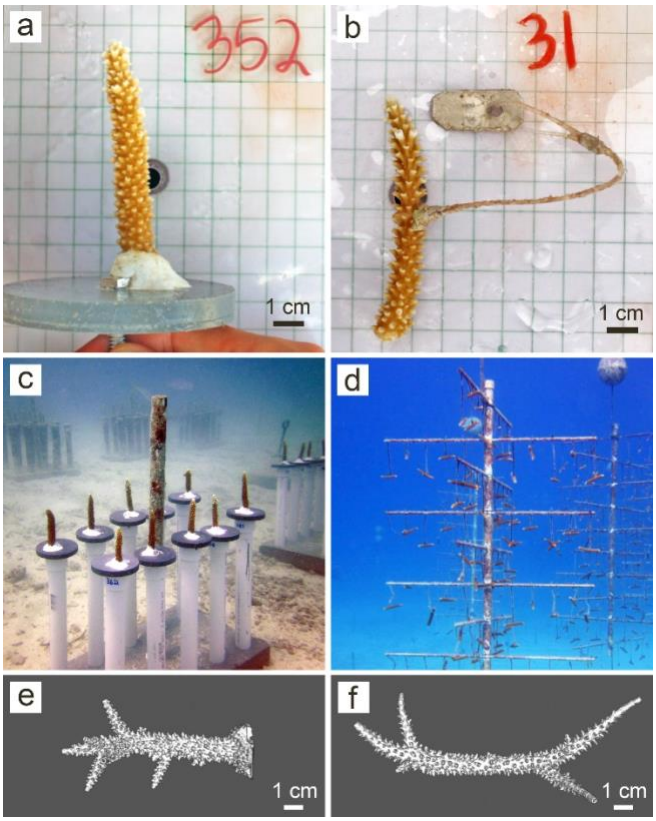
Figure captions

Fig. 1 *Acropora cervicornis* branches (**a**) prepared for deployment on blocks attached to the substratum, (**b**) prepared for deployment on PVC trees in the water column, (**c**) deployed on blocks at the Mote coral nursery, and (**d**) deployed in the water column hanging from PVC trees. Bottom panels (**e**) and (**f**) show the same corals in (**a**) and (**b**) after 191 d of growth scanned with computerized tomography (CT) for measuring skeletal density

Fig. 2 Nursery-raised *Acropora cervicornis* branches reared on blocks attached to the substratum ("block") and hanging from lines in the water column ("water") for 191 d in the summer of 2015. Box and whisker plots show the median value bisecting a *shaded box* representing the interquartile range (IQR), with *whiskers* extending to the minimum and maximum values, and *asterisks* marking possible outliers that are outside the box by more than 1.5x the IQR for (**a**) net total linear extension (TLE; cm), (**b**) skeletal density (g cm^{-3}), and (**c**) calcification rate (mg d^{-1}).

Fig. 3 Calcification rates (mg d^{-1}) of replicate *Acropora cervicornis* branches from eight genotypes subjected to two grow-out methods: attached to plastic disks on cement blocks (*black circles*) or attached to monofilament line hanging from a frame in the water column (*white circles*)



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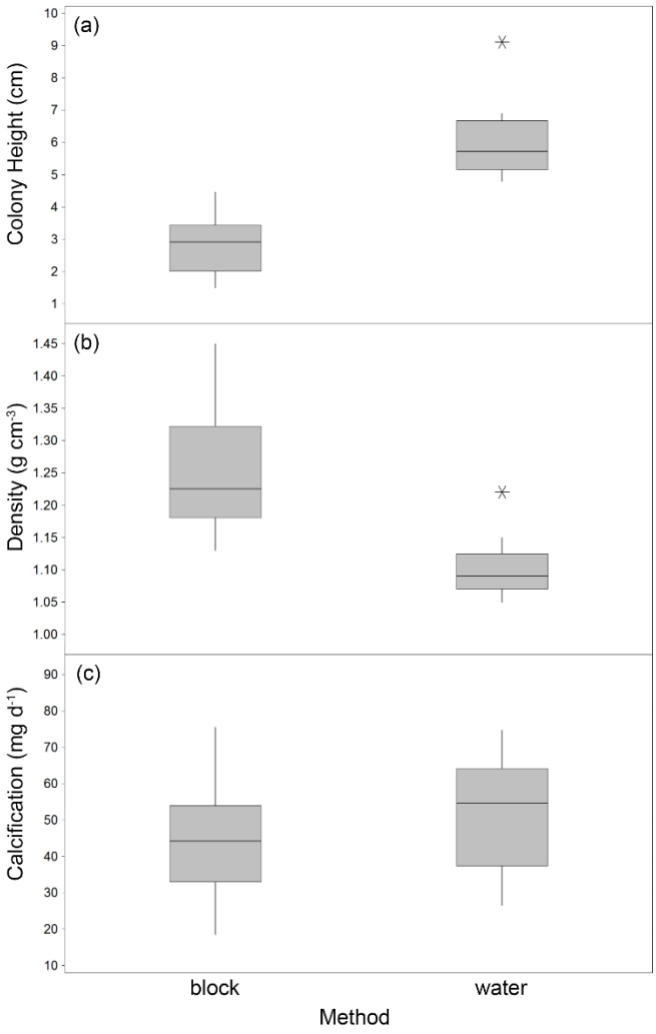

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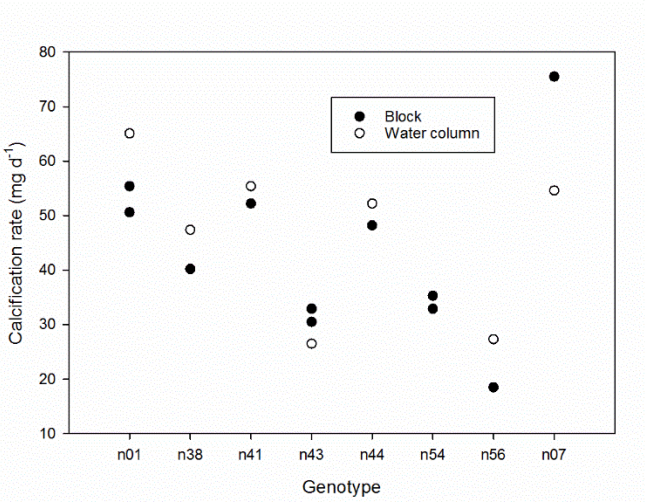

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